Pusat Penelitian dan Layanan Kesehatan Virologi dan Kanker Patologi
Fakultas Kedokteran Universitas Indonesia

25 June 2014

To do list

1. Making LB agar
   - Tools and Material :
     - ➔ Measure tube
     - ➔ 500 mL bottle
     - ➔ Autoclave band
       - Ps = will change to be black stripe if it's sterile
     - ➔ Stirer
     - ➔ Scales
     - ➔ Aquades (dH_2O) , take from cabinet (above)
     - ➔ LB powder (@ chemical lab)
       - 3,5 gr / 100 ml
     - ➔ Petri dish
     - ➔ Autoclave
     - ➔ Micropipette
     - ➔ Alumunium foil

   - Background :
     LB agar is a general medium to grow E. coli on the petri dish.

   - Purpose :
     Prepare the LB agar for MIC

   - Procedure :
     1. Prepare all of the tools and material
     2. Scale the LB powder (3,5 gr for 100 ml agar ; 20 ml/ petri dish)
     3. Mix LB agar powder with dH_2O in 500 mL bottle
     4. Cover it with alumunium foil and sign it with autoclave band
     5. Autoklave it (PS ! Only on 11:00 and 14:00 o'clock)
        a. Put in pass through box DNA room
        b. Put dawn beside the autoclave machine.
     6. Let the LB agar cooling (add antibiotic), (PS: prevent the out of antibiotic order)
     7. Pouring at BSL 2 in r. DNA
        (PS: password "0001"; clean up the bench with alcohol; sign the petri dish on the beside site)

   - Extraordinary occurence
     1. Millard reaction occured on the agar (browny agar, because of too high temperature or repeating autoclave (protocol.org))
     2. Coagulating agar (need faster moves, solution: autoclave again)

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(.....) (.....)

2. MIC

Tabel = variable → control 25, 50, 75, 100, 125, 150, 175

- Background : unknown chloramphenicol MIC

- Purpose : know the MIC of chloramphenicol succinate (equivalent chloramphenicol)

- Tools and material :
  - ➔ LB agar
  - ➔ petri dish
  - ➔ Micropipette
  - ➔ Chloramphenicol 1 gr
  - ➔ Aquades nuclase free 10ml

- Procedure (calculation)

Tabel

	Control OA OB	25	50	75	100	125	150	175
Medium (ml)	20	20	20	20	20	20	20	
Antibiotic (µL)	0	5	10	15	20	25	30	35

PS : dilute 1gr/10ml → 100 mg/ml → 0,1 mg/ml → 100 µg/ µL
25 mg/ml 20 ml = 500mg → 500 µg/(100 µg/ µL) = 5 µL
PS : single treatment to 25 µg/ml because of agar excess

- Procedure
  1. Mix 1 gr chloram with aquades nuclease free 10 ml in antibiotic tube
  2. Make adequte, take1 1,7 mL tube, and save the excess in the refrigerator
  3. Mix with appropriate concentration the we want to the falcon when make the LB agar

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(.....) (.....)

1. Mix LB agar powder with dH_2O in 500 Ml bottle, with ratio 3,5 gr agar LB for 100 ml dH_2O
2. Covered 500 Ml bottle with alumunium foil, pinned autoclave band → autoclave band will be black after the sterilization
3. Autoclave Sterilization Dilution Agar LB in dH_2O
   - Turn on the autoclave machine (autoclave schedule is everyday on 11.00 and 14.00)
   - Import the 500 mL bottle that contain the LB agar to the pass through box.
4. Let the LB agar cooling down (before it rigid)
   - If it was rigid, put LB agar into oven (5 Minutes)
5. Pour the LB agar solution into petri dish
   - Use falcon to amount the volume
   - Add antibiotic using micropippette, then shake agar+antibiotic slowly
   - Do the pouring in BSC

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26 June 2014

1. Make LB Broth (stock) 900ml
   - Background :
     LB broth is used to grow E. coli

   - Purpose :
     Make the stock if we want to culture the E. coli

   - Tools and material :
     Tools
     ➔ 500 mL bottle
     ➔ Magnetic stirer
     ➔ Stirer
     ➔ Autoclave band
     ➔ Scales
     Material
     ➔ Tripton 9 gr, yeast 4,5 gr
     ➔ NaCl
     ➔ dH_2O

   - Procedure :
     1. Mix tripton, yeast, and NaCl with ratio dH_2O (1:0,5:1:0,1) (Tripton:yeast:NaCl: dH_2O)
     2. Pour the dry powder into 500 mL bottle
        ➔ Powder solution measured by scales, pour it to 500 mL bottle, if there's excess use dH_2O
     3. Add dH_2O till 900 ml
     4. Close the bottle and pinned the autoclave band
        ➔ Autoclave band will be black
     5. Autoclave broth, before import into DNA room
     6. Let the LB broth cooling down → put into fridge (kulkas -40^{O}C)

   - Result :
     Transparent yellow LB both

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2. RFP Transformation dan spreading

   - Background
     Resistance chloramphenicol plasmid that contain RFP is inserted to the competent cell, so the transformation will be success or the resistance can be differed from the wild type, with the resistance ability to the antibiotic dosage

   - Purpose
     E. coli mutant which is resistant of chloramphenicol, was tagged by using fluorscent gene, then ready to isolate and replicate

   - Tools
     ➔ Micropipette
     ➔ Microtube 1,7 mL tube 1,5 ml

   - Material
     ➔ Dry plasmid iGEM kit 2013 (3,5 g)
     ➔ dH_2O
     ➔ ice
     ➔ SOC

   - Procedure
     ➔ Add 10 µL dH_2O into dry DNA well, solute in 5 minute (up down)
     ➔ Take 1 µL DNA insert into 50 µL competent cell

   - Result

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27 June 2014

1. Check and Analyze RFP Transformation

   Result :
   A lot of single colony that grow on LB agar, but we can't see the difference of transformation result that we expected.

   Analyze :
   We need to check which is the success RFP transformation to the competent cell. In other hand, there's no competent cell that know wild type transformation, make us must remake the transformation cell with better procedure and modification of IHVCB protocol lab which is appropriated with iGEM protocol.

2. Making LB agar 140 ml (MIC)

   Background :
   LB agar is used for E. coli growing medium

   Purpose :
   Make the stock for MIC E. coli

   Tools
   1. 500 mL bottle
   2. Stirer
   3. Autoclave band
   4. Scales
   5. Alumunium foil
   6. Petri dish
   7. Falcon
   8. micropipete

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(.....) (.....)

28 June 2014

AB Aliquots Status
- ➔ there's difference of antibiotic usage
- ➔ 2 no rigid
  (save in freezer -40^{O}C)

AB (antibiotic)]
- ➔ boiling point 140^{O} – 150 ^{O}C
- ➔ Freezing point -40^{O} sd -50 ^{O}C

DeltaT = (kf/ b) x M x i

Look the MIC result

30 June 2014

1. Made LB agar plate (220 ml) → 11 plate
2. Replication
   a. Tools and material sterilization
   b. LB broth → 40 ml
      (Antibiotic 150, made 10 replica, falcon 10)
   c. Bacteria 150 take 10 colony
      (streak 3 times pakai tusuk gigi,put in to falcon + antibiotic, close)
   d. Streak product on agar replica is incubated in static incubator
   e. Isolation in the broth → is incubated in static inkubator

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1 July 2014

Plasmid isolation

1. Centrifuge 4 ml replica broth with 12.000 g 4^{o}C for10 minutes
2. Pellet is settled

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(.....)	(.....)

6 July 2014

Innoculate the big scale transformation

1. Made LB agar 2 petri dish + chloramphenicol 175 (1,4 gr + 40 ml dH_2O)
2. Calculate bacteria colony
   a. Petri dish 75ng/ml
      196 x 4 = 784
   b. Petri dish 125 ng/ml
      281 x 4 = 1124
   c. Petri dish 150 ng/ml
      320 x 2 = 640
   d. Petri dish 175 ng/ml
      306 x 2 = 612
3. Innoculate big scale (overnight culture)
   LB broth stock

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7 July 2014

Protocol
Plasmid big scale isolation

- ➔ Overnight culture is putted into falcon 50 ml (2 falcon)
- ➔ Centrifuge 3500 rpm 10 menit
- ➔ Discard the supernatant, excess 1 ml (Pelet contain cell + plasmid)
- ➔ Resuspention pelet with excess s/n and move it into 1,7 mL tube
- ➔ Centrifuge 12000 rpm, 1 minute → s/n discard
- ➔ + P1 250 (125) µL, up down
- ➔ + P2 250 (125) µL, invert 4 s.d. 6 x
- ➔ + solution N 3 350 µL, invert 4-6 x
- ➔ Centrifuge 12000 rpm, 10 min → s/n is taken
- ➔ 850 µL → move the supernatan to the spin column based nuclelic acid purification → centrifuge 12000 rpm, 1 minute
- ➔ Discard lysate to the column (repeat it twice → centrifuge)
- ➔ + PB 500 (200) µL → centrifuge 12000 rpm, 1minute → make tha lysate
- ➔ + PE + Etoh 700 (500) µL → (centrifuge 12000 rpm, 1min→ discard the lysate)→ repeat twice
- ➔ Move column into new microtube 1,7 mL
- ➔ + 50 µL 1/3 EB, let it 1 minute
- ➔ centrifuge 12000 rpm, 1 minute
- ➔ + 20 µL 1/3 EB, let it 1 minute
- ➔ centrifuge 12000 rpm, 1 minute
- ➔ (Plasmid in the new microtube 1,7 mL)

    - PE + Etoh → 1 Vol PE : 4 Vol Etoh
    - 1/3 EB → 1 Vol EB in total 3 vol ∑ H_2O
      Ex : 30 µL EB + 60 µL ∑ H_2O

Dilute TE 1x
10 ml → 200 µL 50 x TE + 9800 µL
5 ml → 100 µL 50 x TE + 4900 µL

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(.....) (.....)

50 . x = ½ . 100 ml
X = ½ . 100000 µL / 50
X= 10 µL

Nanodrop

	ng/ µL	Purity	260/230	H_2O
DB 1	72,5	2,03	3,5 µL	12,5 µL
DB 2	80,3	1,94	3,2 µL	12,8 µL

E1	PSB 1	3,5 µL		E2	PSB 1	3,2 µL
	dH_2O	12,5 µL			dH_2O	12,8 µL
	NEBBuf2	2,5 µL			NEBBuf2	2,5 µL
	BSA	0,5 µL			BSA	0,5 µL
	EcoR1	0,5 µL			EcoR1	0,5 µL
	TOTAL	19,5 µL			TOTAL	19,5 µL

Innoculate B. Substilis on petri dish + LB agar
Tools :

1. Petri dish
2. Tips + mcropipet 200 µL

Material :

1. 1,4 gr LB agar
2. 40 ml aquades
3. Bacillus substilis

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8 July 2014

Repeat PLASMID DIGESTION
Preparation running electrophoresis

Tools :
1. Tube cabinet
2. Micropipet
3. microtube 1,7 mL 6

Material :
1. Sample 2 PKS 1 dan PKS 2
2. digestion Enzyme @

RFP 720 bp
pSB1C3 2070 bp

	A	B	C
PKS 1	+EcoRI	+PstI	+EcoRI+PstI
O	O	O	O
PKS 2	+EcoRI	+PstI	+EcoRI+PstI
O	O	O	O

	PKS 1 + digestion Enzyme			PKS 2 + digestion Enzyme		
	A (µL)	B (µL)	C (µL)	A (µL)	B (µL)	C (µL)
DNA (250 ng/ µL)	3,5	3,5	3,5	3,2	3,2	3,2
∑ H_2O	12,5	12,5	12,5	12,8	12,8	12,8
NEB Buffer 3	2,5	2,5	2,5	2,5	2,5	2,5
BSA	0,5	0,5	0,5	0,5	0,5	0,5
EcoRI	0,5	-	0,5	0,5	-	0,5
PstI	-	0,5	0,5	-	0,5	0,5

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(.....) (.....)

8 July 2014

Electrophoresis

	marker	PKS 1	1A	1B	1C	PKS2	2A	2B	2C
9 well									

Tools :

1. Micropipet
2. Glass bottle for agarose + aquades
3. Tips
4. Agarose molding
5. Electrophoresis tool
6. Gel Doc + computer

Material :

1. Agarose 0,8 gr
2. Aquades dH_2O 100ml
3. TAE 0,5
4. Loading dye
5. Ladder (marker)
6. DNA

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10 July 2014

➔ Nuclease from iGEM is coming → save in the room temperature
➔ Plate agar → RFP → moved from incubator to the fridge

11 July 2014

1. Streak and spread E. coli in stab agar from iGEM to LB agar on petri dish
2. Regrow bacteria under petri RFP from incubator
    - Tools
        1. petri dish 4
        2. Micropipet
        3. Falcon 50ml
        4. Tube cabinet
        5. Microtube 1,7 mL tube
        6. Spatel
        7. Tips

    - MATERIAL
        1. Bacateria from iGEM contain plasmid nuclease
        2. Alcohol 70%
        3. LB agar 2,8 gram → 80 ml aquades
        4. Antibiotic → 4 x 35 µL =140 µL

    - Procedure
        1. Scale 2,8 gr LB in chemical lab
        2. Take erlenmeyer, pour the agar into erlenmeyer
        3. Pour 80 ml sterile aquades → cover with alumunium foil
        4. Pinned autoclave band on the alumunium foil
        5. Put erlenmeyer in pass through box
        6. Come in to DNA room → take erlenmeyer LB agar and put into autoclave
        7. Autoclave schedule on 11.00 or13.00
        8. After autoclave, let the agar cooling down
        9. Do the pouring in BSC
        10. Pour the agar into sterile falcon (20mL)
        11. Add chloramphenicol 30 µL
        12. Agar LB + antibiotic → pour into petri dish
        13. Let it until rigid
        14. Streak the bacteria contain chloramphenicol
            a. Devide 10 quadrant (1 quadran1 streak 3 times)
            b. Petri dish packed by parafilm to prevent contamination
            c. Put into static incubator for 1 day
            d. Move it to fridge

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12 July 2014

Take out bacteria from static incubator→ move into fridge.

15 July 2014

Sample		Conc	260/280	260/230
K (-)		-0,9	1,42	0,61
S100	3,4	72,7	1,82	1,33
TolC	6,8	26,6	1,77	1,1
HlyA	3,6	69	1,78	1,34
HlyBD	2,6	96,3	1,59	0,81
RBS	5,2	48,4	1,74	1,34
S119	6,1	40,7	1,8	1,15
D.Term	6	41,6	1,79	1,24
TolC A	4,7	53,2	1,74	1,18
RFP 1		3,2		
RFP 2				
RFP 3				
GFP 4	2,31	13,7		

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18 July 2014

Primer Dilution

- Purpose :
  Dilute the primer

- Background:
  Primer from IDT was in lypolized primer, so we must dilute it

- Tools and material :
  1. Tube
  2. Micropipette
  3. TE buffer pH 8
  4. ddH_2O

- Procedure :
  a. Making master stock (100µM)
     Make the concentration to be 100 µM, for example, if on the tube is written 20,8 nmol, we must add 20,8 x 10 = 208 µL TE buffer, so we get concentration of primer is 100 µM
  b. Making working stock (10µM)
     Dilution formula
     V1.M1=V2.M2
     100.10=V2.10
     V2=100

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19 July 2014

Nuclease

DNA Isolation : Nuclease
Nanodrop

	∑ H_2O		DNA	A260	A280	260/280	
0		1,2		0,024	0,002	11,9	0,38
Kontrol		4,5		0,089	0,031	2,84	0,81
6	9,3	37,3	6,7	0,746	0,833	1,95	1,80
4	10,3	43,2	5,7	0,865	0,438	1,97	1,97
3	12,1	68,5	3,9	1,369	0,714	1,92	2,10
2	9,3	37,3	6,7	0,746	0,365	2,04	1,77

DNA Isolation
26 microtube 1,7 mL
6 spin column based nuclelic acid purification

Digestion
DNA
∑ H_2O
BSA 0,5
NEB Buffer 3 2,5
EcoRI 0,5
PstI 0,5

Electrophoresis
2 agar

Marker	O	Control	Nuc6	Nuc4	Nuc3	Nuc2	Marker
		-	EcoRI	EcoRI	EcoRI	EcoRI	

Marker	Nuc6	Nuc4	Nuc3	Nuc2	Nuc6	Nuc4	Nuc3	Nuc2	marker
	PstI	PstI	PstI	PstI	EcoRI PstI	EcoRI PstI	EcoRI PstI	EcoRI PstI	

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20 July 2014

- PCR Colony running on gell (metode sama dengan elektroforesis biasa)
- Innoculate for DNA isolation in volume 100 ml

Antibitotic concentration that we use: 175 ng/ µL
Final volume 100ml = 10^5
M1 = 100 mg/ml = 100. 10^6 / 10^5 . ng/µL =100. 10^3 = 10^5 ng/µL
V1.M1=V2.M2
V1.10^5=10^5.175
V1=175µL

Making mix marker
DNA ladder 10µL
TE 1x 70µL
6x loading dye 20µL
➔ Total 100µL

Running list
M – Marker – U – S – U – S – Marker 1 – M2 – M3
U= universal (RFP)
S= substilisin

PCR result analysis
RFP colony + universal primer
If it's fail, the running :
➔ Primer was not pinned
➔ Wrong primer dilution (TE)
➔ PCR is hold on 15 hours in 0^OC
➔ Minimum amount of colony

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21 July

DNA Isolation
K-
Nuc4
Nuc6

Nanodrop	ng/ µL	A260	A280	260/280	260 620	DNA	∑ H_2O
K-	6,0	0,121	0,062	1,94	0,41	41 ~ 16	
Nuc4	148,9	2,979	1,490	2,0	1,83	1,67 ~ 1,7	14,3
Nuc6	101,3	2,026	1,058	1,92	1,52	2,46 ~ 2,5	13,5

BSA 0,5
NEB Buffer 3 2,5
EcoRI 0,5
PstI 0,5

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24 July 2014

PCR

Extraordinary occurence :

1. Failed to make PCR mix

Make culture in LB Broth → falcon 50 ml
Grow B. Substilis → for DNA isolation
Incubate → shaker incubator

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25 July

1. Make TEN reagent for B. Substilis DNA isolation
2. DNA Plasmid isolation from B. Substilis by heating

a. Take away overnight culture B. Substilis from shaker incubator
b. Take it
c. → heating in 100^{O}C for 10 minute PCR machine
d. Take it

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26 July 2014

Running PCR Result

Extraordinary occurence :
- Yesterday agar was putted on electrophoresis chamber
- Lefted overnight → too soft, fricted agar, running was too fast band reach the border

Making 2 more agar
1. Substilis
2. Universal

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28 July 2014

1. MalS Transformation to the Top10
2. Stocked in -80^{O}C
3. HlyA Transformation in LB broth

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30 July 2014

Learn new protocol → PCR : Hot Star !
Protocol → volume of mix PCR differrent with dream taq method
We use hot star PCR because of failure past PCR method (dimer)

Dimer
- ➔ forward Primer with reverse primer
- ➔ forward Primer with forward primer

The characteristic of dimer : After PCR running, ther will be no underline on the top, but there's a thick sign at the under.

Touch down
- Not only use hot star method to prevent the dimer, but we also do the touch down / temperature optimation→ use 10 column
  Gradient of temperature only 50-70^{O}C

Column	Temperature (^{O}C)
1	50
2	50,5
3	51,7
4	53,2
5	55,5
6	58,4
7	61,8
8	64,6
9	66,8
10	68,4
11	69,6
12	70,0

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30 July 2014

Protocol : Hot Star (12 reaction)

Volume total	125	µL (PCR Mix)
10 x taq buffer	12,5	µL (PCR Mix)
5 x solution $MgCl_2$ 25 Mm	25,00	µL (PCR Mix)
dNTP 10 mM	2,5	µL (PCR Mix)
Primer mix (F t r)	6,25	µL
Taq Hot Star	0,63	µL
Template	6,25	µL
∑ H_2O	71,88	µL (PCR Mix)

- Running PCR result → HlyA + Nuclease → PCR banding dreamtaq + hotstar
- Transform HlyA (-PCR), MalS (+PCR), RFP (+)
- Substilisin → protocol of lysis = make the pellet! not supernatant → PCR dreaming hotstar

50 µL competent cell + genofinterest

+RFP, control (+)

control (-) Mo → LB +antibio, +top 10 Wt

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31 July 2014

100 x TAE = 0
→500 → 2,5 mL TAE 100X → 500 mL – 2.5 = 497,5

50 x V = 0,5 X 500
V = (0,5 x 500)/50
v = 5 mL → 500 mL

Making LB Agar
300 amL aquades + 10,5 LB agar.
→ make 7 plate
2 RBS + @20 µL amphicilin
2 MalS + @40 µL chloramphenicol
2 RFP + @40 µL chloramphenicol
1 control (-)

Transformation of MalS, RFP, RBS.
MalS = 2013 plate 1 well 4P
RBS = 2014 plate 4 well 1N
RFP = Plasmid from DNA isolation

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31 July 2014

Transformation Protocol (Modified by Yuda)

1. Prepare the competent cell 50 µL
2. Add 1 – 2 µL DNA from kit
3. Incubate for 60 minutes in ice
4. Heat shock on 42^0 C for 90 second
5. Incubate in ice 4^0 C 5 minute
6. Add 200 µL 50 C media
7. Incubate in shaker 2-3 jam
8. Plating → 200 ng / µL add 40 µL (kloramfenikol), 20 µL (amphicilin)

Amphicilin stock concentration 100 mg/mL

MIC = 1000 mL = 1 mL

m1 x v1 = m2 x v2
100 mg/mL x 1 mL = 1000 mL x M2
M2 = 0,1 mg/mL

0,02 mL= 20 µL
100 v1 = 0,1 x 20
v1= 0,02 mL
v1= 20 µL

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31 July 2014

Running Check Marker ✓
Running HlyA positive✓
Running Universal works ✓

1 August 2014

- DNA isolation B subtilis Rao Method
- Check transformation
- PCR substilis
  - malS was failed to be tranformed because we use kit 2013

1 August 2014

- HlyA, malS, HlyDB Transformation
- Isolation B. Substilis. Rao Method

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4 August 2014

1. Boiling dan Sonication
   Purpose : Get DNA genome

   Boiling :
   1. Bacteria is grown in LB and then shake 200 rpm overnight
   2. Move it into microtube 125 ml, centrifuge 12.000 rpm 5 minute
   3. Take the pellet and add 1/3 TE rinse 3x
   4. Heat 100^0 C 10 minute
   5. Sonication
      → 20 seconds do the sonication
      → 10 seconds incubate in ice
      repeat 6 times for the 5th step
   6. Centrifuge 12.000 rpm 4^0 C , take supernatant

Transformation	Well	Plate
Chez	18 G	1
GFP	13 L	4
R rose	15 A	4
R for LI		

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Loading dye 6x

m1.v1 = m2.v2
1.v1 = 6.mL
= 6 mL

TE = 5
1 X = 1/ 6 Ml

V1. M1 = V2.M2

5 ml LB broth Amphicilin 5 µL
5 ml LB broth Chloramphenicol 10 µL

Transformation	Well	Plate
CheZ		
GFP		
RNA rose		
RNA forU		
Cph8	15 P	2
PcyA	13 H	3
Ho7	17 P	3
OmPR promoter	3 I	3
Tol C	40	1

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5 August 2014

Nanodrop

No.	Sample	Concentration	260/280	Volume DNA yang dipakai
1	HlyA	49 ng/ µL	1,97	5,2 µL
2	HlyD	76,3 ng/ µL	1,84	3,3 µL
3	Strong Promoter	84,7 ng/ µL	1,85	3,0 µL
4	RBS	43,9 ng/ µL	1,94	5,7 µL
5	MalS	100,8 ng/ µL	1,89	2,5 µL

Resep Digesti

No.	Sample	DNA	dH_2O	NEB Buffer 2	BSA	EcoRI	Total
1	HlyA	5,2	10,8	2,5	0,5	0,5	19,5
2	HlyD	3,3	12,7	2,5	0,5	0,5	19,5
3	Strong Promoter	3,0	13,0	2,5	0,5	0,5	19,5
4	RBS	5,7	10,3	2,5	0,5	0,5	19,5
5	MalS	2,5	13,5	2,5	0,5	0,5	19,5

1. Marker 2,3 RBS 4,5 HlyA 6,7 MalS 8,4 HlyD 10,11 Strong Promoter

Nuclease : M-1,2,3,4,5-M-10,9,8,7,6-M

Isolasi Besar.

1. RBS
2. MalS
3. RFP
4. HlyA
5. HlyD,B
6. Nuclease
7. Strong Promoter

Testified by

(.....)

Supervisor

(.....)

Testified by	Supervisor
(.....)	(.....)

6 August 2014

Nuclease DNA Isolation

Nanodrop Nuclease DNA Isolation

Number	Nanodrop	mg/ µL	A260	A280	260/280	260/320
1	R-	-	-	-	-	-
2	Nuclease 1	84,5	1,689	0,914	1,85	1,34
3	Nuclease 2	109	2,179	1,162	1,88	1,73

Mixing PCR
Hotstar 12 cycles (substilis)

1. Volume total 125 µL (12 siklus)
2. 10 x taq buffer 12,5 µL (PCR Mix appendorf)
3. 5 x solution $MgCl_2$ 25 mM 25,00 µL (PCR Mix appendorf)
4. dNTP 10 mM 2,5 µL (PCR Mix appendorf)
5. Primer mix (F + R) 6,25 µL
6. Taq Hot Star 0,63 µL + M13 + PKS
7. Template 6,25 µL
8. ∑ H2O 71,88 µL (appendorf PCR Mix)

Protocol PCR Hotstar taq

Temperature	Time	Cycles
95^0 C (preheat)	15 minute	1 x
Denaturation **94^0 C**	0,5 s.d. 1 minute	35-40 x
*Annealing **50-68^0 C**	0,5 s.d. 1 minute	
Elongation **72^0 C	1 minute	
Last heating	10 minute	1 x

*Tm – 5^0 C
**Elongation DNA waktu : 1 kb/ minute
Storage : 20^0 C

Testified by Supervisor

(.....) (.....)

6 August 2014

Boiling dan Sonikasi Method V.2

1. Spin 500 µL decontaminated sample - for 15 minute
2. Centrifuge 10000 x gmg
3. Discard the supernatant
4. Take the pellet and add 100 µL DNAase free water (∑ H2O)
5. Incubate for 20 minutes 95^0C and boil it in waterbath
6. Sonication 15 minute
7. Centrifuge 5 minutes full speed 13000 → take the supernatant
8. Use 5 µL supernatant for PCR template

MIXING PCR
Dream taq 5 cycles

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Supervisor

(.....)

6 August 2014

Repeat the innoculation and streak replica of transformation 1st
Repeat the innoculation and streak replica of transformation 2nd

List colony transformation 1st :

1. MalS ✓
2. RBS ✓
3. RFP✓
4. Nuklease → have been isolated today
5. Strong promoter✓
6. HlyA✓
7. HlyB,D✓

List colony transformation 2nd:

1. CheZ ✓
2. GFP ✓ *Amphicilin
3. RNA rose ✓
4. RNA forU ✓
5. Cph8 ✓
6. PcyA ✓
7. HO7 ✓
8. OmpR promoter ✓
9. TolC ✓

Testified by

(.....)

Supervisor

(.....)

Streak replica → LB agar

Plate	Plasmid	Antibiotic
1	RRS + GFP	chloramphenicol
2		
3		
4		
5		
6		
7		
8		

Testified by

(.....)

Supervisor

(.....)

7 August 2014

Isolation result
Nanodrop : HlyA 1-5, Mol 5 1-5, RFP 1-5, HlyB,D 1-5

Number	Sample	DNA	Concentration (ng/ µL)	∑ H2O	A260	A280	260/280	260/230
1	K (-)		0,2		0,0004	0,0004	1,07	1,88
2	HlyA 1	26,5	9,4	6,6	0,188	0,094	1,89	2,38
3	HlyA 2	0,8	307,6		6,151	2,601	2,20	2,37
4	HlyA 3	12,5	20,0	3,5	0,400	0,207	1,93	1,86
5	HlyA 4	9,05	27,6	6,95	0,552	0,261	2,12	1,46
6	HlyA 5		0,1		0,002	0,004	0,46	0,1
7	MalS 1	6,5	38,4	9,5	0,768	0,383	2,00	1,94
8	MalS 2	3,25	76,8	12,75	1,536	0,815	1,88	2,22
9	MalS 3	3,23	77,2		1,544	0,817	1,89	1,79
10	MalS 4	8,38	29,8	7,62	0,596	0,310	1,92	1,79
11	MalS 5	5,19	48,1	10,81	0,963	0,499	1,93	1,61
12	RFP 1	4,1	59,8	11,9	1,195	0,592	2,02	2,17
13	RFP 2	1,9	177,5		3,550	1,846	1,92	2,20
14	RFP 3	2,36	105,8		2,115	1,092	1,94	2,04
15	RFP 4	1,90	131,3	14,1	2,626	1,402	1,87	2,22
16	RFP 5	3,99	62,6	12,01	1,251	0,645	1,94	2,17
17	HlyBD 1	5,89	42,4	10,11	0,874	0,427	1,98	1,38
18	HlyBD 2	8,25	30,3		0,605	0,304	1,46	1,77
19	HlyBD 3	9,46	26,4		0,527	0,276	1,41	1,94
20	HlyBD 4	18,3	13,6		0,272	0,130	2,09	1,65
21	HlyBD 5	6,52	38,3	9,48	0,767	0,395	1,94	1,52

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8 August 2014

Num	Sample	DNA	Concentration (ng/ µL)	∑ H2O	A260	A280	260/280	260/230
1	K (-)		0,1		0.002	0,010	0,20	1,22
2	St. Promoter 1	8,5	29,4		0,589	0,291	1,49	1,87
3	St. Promoter 2	9,15	37,3		0,746	0,381	1,95	1,84
4	St. Promoter 3	8,79	28,6		0,573	0,274	2,09	1,89
5	St. Promoter 4	2,63	94,7	13,37	1,894	0,995	1,90	1,99
6	St. Promoter 5	9,208	59,4	11,7	1,188	0,617	1,92	1,82
7	OMPR 1	7,12	35,1	8,8	0,702	0,352	1,99	1,85
8	OMPR 2	4,1	60,8	11,9	1,216	0,635	1,91	2,05
9	OMPR 3	6,8	36,5		0,730	0,364	2,01	1,94
10	OMPR 4	7,7	32,2		0,644	0,319	2,02	1,76
11	OMPR 5	8,3	30,1		0,602	0,302	2,00	1,80
12	HO7 1	4,17	59,9	11,83	1,199	0,626	1,91	2,14
13	HO7 2	9,6	25,8		0,516	0,255	2,02	1,95
14	HO7 3	11,6	21,5		0,430	0,192	2,23	1,92
15	HO7 4	5,28	47,3	10,72	0,445	0,491	1,93	2,04
16	HO7 5	15,7	15,9		0,317	0,141	2,25	1,87
17	RNA rose 1	5,9	42,1	10,1	0,842	0,411	2,05	1,61
18	RNA rose 2	4,72	52,9	11,28	1,059	0,586	1,98	1,91
19	RNA rose 3	8,74	28,6		0,572	0,260	2,20	1,79
20	RNA rose 4		27,9		0,557	0,262	2,13	1,64
21	RNA rose 5		13,2		0,265	0,109	2,42	1,38

Note : (Tol C 5, CheZ 4, CheZ 5, Rose 5) was missing, CPH8 only 2

Testified by

(.....)

Supervisor

(.....)

No.	Sample	DNA	Concentration (ng/ µL)	∑H2O	A260	A280	260/280	260/230
1	K (-)		0,4		0,807	0,0002	-4,35	-2,93
2	RBS 2	14,97	16,7	1,03	0,334	0,175	1,90	1,61
3	RBS 3	3,931	63,6	12,069	1,272	0,681	1,87	1,81
4	RBS 5	9,58	26,1	6,42	0,522	0,270	1,93	1,81
5	RBS 4		102,2				1,83	1,09
6	TolC 1	9,857	27,3	6,843	0,545	0,286	1,91	1,52
7	TolC 2	3,444	72,6	12,556	1,452	0,770	1,88	2,00
8	TolC 4	4,386	57,0	11,6			1,93	1,68
9	GFP 4	2,31	108,2	13,7	1,141	0,589	1,86	2,15
10	GFP 3	4,55	55,0	11,45	1,101	0,568	1,94	1,81
11	GFP 5	5,28	47,3	10,72	0,946	0,490	1,93	1.85
12	RNA Rose 1	10,4	24,0	5,58	0,480	0,252	1,91	1,73
13	CheZ 1	3,99	62,7	12,0	1,254	0,664	1,89	2,07
14	CheZ 2	4,8	52,1	11,2	1,043	0,552	1,89	1,90
15	CheZ 3	4,31	58,0	11,69	1,160	0,626	1,85	1,78
16	RNA Rose 3	3,0	50,4	11			1,92	1,76
17	RNA Rose 5		37,1				2,01	1,67
18	Cph8 1	2,56	97,6	13,44			1,91	1,95
19	Cph8 2	9,3	26,9	6,7			1,08	1,11
20	Cph8 3	3,28	76,2	12,7			1,91	1,94
21	Cph8 4	3,7	67,3	12,3			1,90	1,68
22	Cph8 5		40,2				1,94	1,77
23	PcyA	4,2	59,5	11,8			1,91	1,89
24	PcyA	4,93	50,7	11,07			1,95	1,80
25	PcyA	7,08	35,3	8,92			2,05	1,04
26	PcyA	4,17	59,9	11,83			1,92	1,78
27	PcyA	4,1	61,1	11,9			1,92	1,98
28	RFP 4	1,81	130,1	14,2			1,80	1,93
29	RFP5	2,16	125,7	13,84			0,94	0,61

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Supervisor

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8 August 2014

G Blocks
Ligase

1. Dilute TE 20 µL
- Concentration 200 ng/20 µL = 10 ng/ µL

2. Take 10 µL (100 ng) → double digest with Eco RI and pstl

Protocol	gBlocks	Vector (RFP)
DNA	10 µL	250 ng → 4 µL
dH_2O	6 µL	12 µL
NEB2	2,5 µL	2.5 µL
BSA	0,5 µL	0.5 µL
EcoRI	0,5 µL	0.5 µL
Pstl	0,5 µL	0,5 µL
Digested	20 µL	20 µL
	Inserted	Vector

3. PCR machine
   37^0 C 30 minute + 80^0 C 20 minute

4. Take 4 µL (20 ng) digested insert (untuk ligasi)
   Take 4 µL (50 ng) digested vector (untuk ligasi)

Protocol	
digested vector	4 µL
digested insert	4 µL
T4 buffer	1 µL
T4 ligase	0,5 µL
H_2O	0,5 µL +
	10 mL

5. PCR machine → 23^0C 2 hours (16^0C 16 hours) + 80^0C 20 minutes

6. Take 4 µL → for transformation

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(.....)

8 August 2014

Lab Plan 8 August 2014

1. Continue 8 genes isolation → 40 falcon
   → There's only 7 genes, except Nuclease, so it must be 35 falcons, but 3 had missed when washing, so there are 32 falcons left

2. Nanodrop in the next 2 pages
   DNA isolation result → aliquot → digestion

3. Running electrophoresis → digestion result
   →2 @1 part → take 1 (inoculate in LB Broth 100 ml)

   Making LB Broth 1000 ml
   Plan priority parts 2
   - MalS
   - Strong Promoter
   - HlyA
   - HlyBD
   - RBS
   - TolC
   - RFP

4. PCR → touchdown MalS (DT) → running electrophoresis

5. Restriction ligation of peptide 1018 + RFP vector

Testified by

(.....)

Supervisor

(.....)

Testified by	Supervisor
(.....)	(.....)

9 August 2014

1. DNA isolation MalS and Strong Promoter (after 12 o'clock → 2 hours)
2. Running digestion result (8 August 2014) (11 s.d. 12)
3. Digest DNA isolation result 2nd (11 s.d. 12)
4. Transform ligation result : peptide 1018 gBlocks (after 12 o'clock → 3 hours)
5. *B. substilis* isolation (3 hours)

Running (8 August 2014)
Marker MalS MalS St. prom St. prom 1 2 3 4 m

Running (9 August 2014)
Marker(old). $HO7_1$ $H07_4$ $RNAFU_1$ $RNAFU_2$ RFP_4 RFP_5 $OMPR_1$ $OMPR_2$ $HlyB_1$ $HlyB_5$ $HlyA_3$ $HlyA_4$

Marker(old) $MalS_2$ $MalS_4$ St. $prom_4$ St. $prom_5$ (PCR $MalS_1$) (PCR $MalS_3$) (PCR $MalS_5$) Marker(baru)

PCR Hifi PFX mix	(Nuclease) µL	(MalS) µL
10 x PFX	1,3	1,3
10 X PCR enhance	1,3	1,3
10 mM dNTP	0.39	0,39
50 mM $MgSO_4$	0,26	0,26
Primer 10 µL	0,39 (VF2 & VR)	0,39 (VF2 & MalS reverse)
Platinum PFX	0,1	0,1
∑ H2O	8,26	8,26
Template	1,3	1,3

Program PCE machine
PCX Hifi

Testified by Supervisor

(.....) (.....)

9 August 2014

Digestion, Nanodrop
PCR Hifi Nuclease

Program protocol PFX Hifi (Nuclease, MalS) → Primer Universal Tm 58

	Temperature	Time	Cycles
Initiation Denaturation	94^0 C	3 minute	1 x
Denaturation	94^0 C	30 second	40 x
Annealing	opt(53^0 C)	30 second	
Elongation	72^0 C	1000 bp/min (2mm)	
Last Elongation	72^0 C	10 minute	1 x

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(.....)

Supervisor

(.....)

11 August 2014

1: - MalS DNA isolation
- Subtilis PCR
- Digestion

2 : - Nanodrop
- make the Plate → spread transform
Transform peptide 1018, added parts

Transformation kitplate 2014

Parts	Plate	Well	Antibiotic
Strong Promoter 100	4	17D	Amphicilin
Strong Promoter 119	4	17B	Amphicilin
Peptide 1018	-	-	Chloramphenicol
Double terminator	3	3F	Chloramphenicol
Lambda represor	2	4B	Chloramphenicol
Lambda Promoter	4	5L	Amphicilin

→ PCR DT → CDLony PCR
HlyA, RFD → primer = VF2, VR
MalS → VF2, MalS R

→Running PCR

Testified by

(.....)

Supervisor

(.....)

Testified by	Supervisor
(.....)	(.....)

11 August 2014

Electrophoresis

Marker; RNA Rose$_2$;RNA Rose $_3$;RBS B$_2$;RBS B$_3$;RBS B$_5$;GFP$_3$;GFP$_4$;PcyA$_4$;PcyA$_5$;CPH8$_3$;CPH8$_4$

Bacillus Substilis

Column 3 , 5 , 7 , 9 , 11

PCR date 14 August 2014
List

I. CheZ1, CheZ2, CheZ3| GFP1, GFP2, GFP3 | RFP1, RFP2, RFP3| RNAR1, RNAR2, RNAR3|
720 706

II. LR1, LR2, LR3 | OMPR1, OMPR2, OMPR3 | HBl1, HBl2, HBl3| CPH81, CPH82, CPH83|
RNAF1, RNAF2, RNAF3|

III. PCY81, PCY82, PCY83

Testified by Supervisor

(.....) (.....)

12 August 2014

To do List Lab Target

1. making PQE 80L stock has done
2. running elektroforesis taq Hifi (11 August 2014)
3. streak bacteria target 6
    1. RB5 (Amphi)
    2. HlyB,D (Chloram)
    3. TolC (Chloram)
    4. HlyA (Chloram)
    5. Double Terminator (Chloram)
    6. St. Promoter 100 (Amphi) + 7 St. Promoter 119 Amphi 8 RFP
    7. MalS (chloram)
    8. Nuclease (chloram)
4. Isolate substilis → PCR (Hotstar) ; Substilisin
5. mix PCR for PCR colony DT → Hifi

Testified by

(.....)

Supervisor

(.....)

12 August 2014

Prepare agar in petri dish → make paper background
Make for 12 µL ∑ H_2O + bacteria colony
Innoculate 1 colony, and shake until the bacteria in ∑ H_2O 12 µL

RFP → is taken 3 colonyes

1. RBS
2. TolC
3. HlyB
4. RFP
5. HlyA
6. D. Term
7. S. prom 100
8. S. prom 119
9. MalS
10. Nuclease

(Total : 30 PCR tubes contain template)

Testified by

(.....)

Supervisor

(.....)

13 August 2014

Running Electrophoresis (wian + hanifi)

Marker
RBS 1
RBS 2
RBS 3
TolC 1
TolC 2
TolC 3
HlyB 1
HlyB 2
HlyB 3
HlyA 1
HlyA 2
Marker

Marker
HlyA3
Double terminator 1
Double terminator 2
Double terminator 3
S100 1
S100 2
S100 3
S119 1
S119 2
S119 3
RFP
Marker

PCR DT
PCR Hifi
TolC 3
RBS 3
St. Prom 119 3
St. Prom 100 1
Double terminatot 1
HlyA 3
HlyB,D 1

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(.....)

Supervisor

(.....)

		B4		B4		B4		B4		B4	
			B2				B2				
		KoI		KoI		KoI		KoI		KoI	

Dream Taq
PCR mix dream taq → 50 µL= 5 reaction

10 x Dreamtaq Buffer	5 µL
2 . 10 mM dNTP	5 µL
Primer Forward	0,6
Primer Reverse	0,6
Dream taq polymerase	1,25
∑ H_2O	...

Program PCR dreamtaq	Temperature	Times	Cycle
Initial denaturation	95 ^{O}C	5 minute	1
Deanturation	95 ^{O}C	30 s	25-40
Annealing	(opt) 53 ^{O}C	30 s	
**Extension/elongation	72 ^{O}C	1000 bp/min	
Final extension	72 ^{O}C	10 minute	1

* Tm = X ^{O}C
** Annealing = X ^{O}C - 5 ^{O}C

Testified by

(.....)

Supervisor

(.....)

14 August 2014

Lab Target

1. DT
2. MalS
3. Nuclease
4. RFP
5. OMPR
6. GFP
7. CheZ
8. RNA rose
9. Cph 8
10. PcyA
11. H07
12. RNA for U

Testified by

(.....)

Supervisor

(.....)

15 August 2014

Electrophoresis PCR DT result
Take out the transformation

HlyBD DNA isolation
Nanodrop

Sample	Concentration (ng/µL)	A60	A280	260/280	260/230
HlyBD 1	127,8	2,55	1,225	2,09	2,08
HlyBD 2	317,9	6,359	2,969	2,14	2,25

Testified by

(.....)

Supervisor

(.....)

16 August 2014

1. prepare Vibrio growth medium
2. do the DNA isolation Peptide 1018 & T5 promoter
3. nanodrop → digestion

1,7 mL tube used : 8
Spin column based nuclelic acid purification used : 8

Testified by

(.....)

Supervisor

(.....)

	1018 1	1018 2	1018 3	1018 4	1018 5	T5 1	T5 2	T5 3	HlyB 1	HlyB 2
DNA										
∑ H_2O										
NEB 3	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
BSA	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
PstI	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5

Sample	Concentration	A260/280	260/230
1018 1	97,6	1,93	1,81
1018 2	338,1	1,95	1,88
1018 3	96,0	1,97	1,49
1018 4	96,7	1,86	1,15
1018 5	149,5	2,01	1,84
T5 1	178,9	1,99	1,80
T5 2	107,7	1,93	1,57
T5 3	76,9	1,86	1,24

Peptide 1
- Take → 2,56µL
- ∑ H_2O → 13,45 µL

Peptide 3
- Take → 2,6 µL
- ∑ H_2O → 13,4µL

Peptide 4
- Take → 2,6 µL
- ∑ H_2O → 13,4 µL

NEB Buffer 3 → 2,5 µL x 6 = 15 µL
BSA → 0,5 µLx 6 = 3 µL
PstI → 0,5 µL x 6 = 3µL

Testified by

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Supervisor

(.....)

DreamTaq

10x buffer	5 µL
dNTP	5 µL
Fwd	5 µL
Reverse	5 µL
H_2O	29,75 µL
DreamTaq	0,25 µL
Template	2 µL
TOTAL	50 µL

PCR Test

1	Subs + bac. Subs	10
2	Univ + plasmid kit	5
3	Univ + red codon	5
4	Subs + no DNA	10
5	Univ + no DNA	5
6	M13 + PKS	7

10x buffer	5 µL X 6
dNTP	5 µL X 6
Fwd	5 µL
Reverse	5 µL
H_2O	29,75 µL X 6
DreamTaq	0,25 µL
Template	

Testified by

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Supervisor

(.....)

	Peptide 1018	Linear pSB1C3	RFP
DNA	4 µL	10 µL	
dH_2O	12 µL	6 µL	
NEB 3	2,5 µL	2,5 µL	2,5 µL
BSA	0,5 µL	0,5 µL	0,5 µL
EcoRI	0,5 µL	0,5 µL	0,5 µL
PstI	0,5 µL	0,5 µL	0,5 µL
DpnI	-	0,5 µL	-
Total	20 µL	20,5 µL	20 µL

Testified by

(.....)

Supervisor

(.....)

19 August 2014

PCR Hifi MalS Nuclease

Testified by

(.....)

Supervisor

(.....)

20 August 2014

Ligase digestion result
Transform ligation result → try BL21 CP – RFP
Consultation and discussion with dr. Budi
Heat MalS, Nuclease

Assembly Step1 (and transform)

1. S100 + RBS (K)
2. S119 + RBS (K)
3. T5 + RBS (A)
4. 1018 + HlyA (A)
5. TolC + HlyBD (A)
6. S100 + Rose (A)
7. RBS + GFP (K)
8. RFP Amphi Backbone (A)
9. MalS + HlyA (no transform)
10. Nuclease + HlyA (no transform)
11. Substilisin + HlyA (no transform)
12. RBS + 1018 (A)
13. RBS + CheZ (A)
14. RFP + GFP Backbone (A)

Testified by

(.....)

Supervisor

(.....)

21 August 2014

Isoamyl alcohol method → genome & substilis isolation
Transformation result → incubator
10 → a bit growth of colony, small diameter, RFP was not expressed
13 → idem
BL21 CP → not turn red
TolC → HlyBD ✓
10 plate (3 chloram + 7 amphi)

Sample	Concentration	A260	A280	260/280	260/230
Blank	-0,1	-0,002	-0,011	0,15	-0,2
MalS	39,4	0,788	0,540	1,46	0,49
MalS	40,5	0,810	0,561	1,44	0,49
Nuclease	24,8	0,497	0,331	1,50	0,55
Nuclease	24,9	0,497	0,329	1,51	0,55

Testified by

(.....)

Supervisor

(.....)

22 August 2014

Sp100-RBS 3
S119-RBS 6
RBS-GFP 1
TolC-HlyBD 3
T5-RBS 3
1018-HlyA 3
Sp100-Rose 2
RFP-GFP 3
RFP-Amphi 1
RBS –cheZ 3

TolC

Testified by

(.....)

Supervisor

(.....)

23 August 2014

Digestion

- 1018 (3) DNA 2,6 ul + H_2O 13,4 ul
- RBS Hifi (48,4 ung/ul) DNA 5,2 ul + H_2O 10,8
- Linear Amp DNA 10 ul + H_2O 6 ul
- MalS Hifi new (130 ng/ul) DNA 2 ul + H_2O 14 ul
- Nuclease Hifi new (133 ng/ul) DNA 1,9 ul + H_2O 14,1 ul
- HlyA hifi DNA 3,6 ul + H_2O 12,4 ul
- RFP 2 (82,8 ng/ul) DNA 3 ul + H_2O 13ul

Nanodrop Purified PFX Hifi MalS Nuclease

Sample	Concentration (ng/ul)	A260	A280	260/280	260/230
Blank	0,5	0,01	0,013	0,72	0,61
MalS (1)	130,3	2,605	1,391	1,87	2,15
MalS (2)	133,6	2,672	1,430	1,87	2,07
Nuc (1)	133,8	2,675	1,441	1,86	2,1
Nuc (2)	138,9	2,777	1,492	1,86	2,09

PFX Hifi, success! (DNA concentrarion is high, Running electrophoresis: suitable length band)

LAB PLAN IGEM 2014

Making LB Broth stock 1L = devide 2 (500,500)
running PFX Hifi MalS, nuclease
Purify PFX Hifi MalS, nuclease
Running electrophoresis PCR colony
Confirmation → DNA isolation
DNA isolation
Nanodrop isolation
Digest, Ligase, Transform (3 Transformation)

1. P1018 – RBS
2. MalS – HlyA
3. Nuc – HlyA

DNA isolation list

- TolC HlyBD 2
- TolC HlyBD 3
- P1018 HlyA 2
- P1018 HlyA 3
- RFP Amphi 1
- Sp100 RBS 2
- Sp100 RBS 3
- T5 RBS 2
- T5 RBS 3
- Sp100 Rose 1

Testified by Supervisor

(.....) (.....)

- Sp100 Rose 2
- RFP GFP 1
- RFP GFP 3
- RBS CheZ 1

Isolation result
Nanodrop

Sampel	Conc (ng/ul)	A260	A280	260/280	260/230
Sp100-Rose 1	90,5	1,809	0,895	2,02	1,9
Sp100-Rose 2	63,7	1,275	0,644	1,98	1,83
T5-RBS 2	70,9	1,419	0,713	1,99	1,89
T5-RBS 3	93,3	1,867	0,917	2,04	1,96
RFP-GFP 1	111,8	2,237	1,110	2,01	1,89
RFP-GFP 3	61	1,221	0,622	1,96	1,77
	66,8	1,336	0,746	1,79	2,29
RBS-CheZ 1	88,9	1,779	0,885	2,01	1,92
TolC-HlyBD 2	125,8	2,517	1,253	2,01	2,08
TolC-HlyBD 3	135,8	2,716	1,34	2,03	2,06
	133,1	2,661	1,31	2,03	2,04
P1018-HlyA 2	165,8	3,316	1,66	2	1,95
	150,6	3,012	1,518	1,98	1,92
P1018-HlyA 3	125,2	2,504	1,28	1,96	2,01
	111,1	2,22	1,135	1,96	1,93
RFP Amphi 1	176,3	3,525	1,84	1,92	2,11
	176,4	3,529	1,849	1,91	209
RFP cheZ 3	88,6	1,772	0,901	1,97	1,99

Running Elektroforesis
Well 1

1. S100-Rose 1
2. S100-Rose 2
3. T5-RBS 2
4. T5-RBS 3
5. RFP-GFP 1
6. RFP-GFP 3
7. RBS-CheZ 1

Well 2

1. RBS-CheZ 3 → 2000
2. 1018-HlyA 2 → 2000-2500
3. 1018-HyA 3 → 2000-2500
4. RFP amphi 1 → 3000
5. TolC-HlyBD 2 → 2000-2500
6. TolC-HlyBD 3 → 2000-2500

SP Rose

S100-PCR
Rose-isolation
T5-isolasi
RBS-Hifi
CheZ-isolation

Testified by | Supervisor

(.....) (.....)

1018-isolation
HlyA-PCR
TolC-PCR
HlyBD-isolation

Testified by

(.....)

Supervisor

(.....)

24 August 2014

DNA isolation

	Conc	260/280	260/230
S100- RBS 2	43,4	1,86	1,76
RBS-GFP 1	73,6	1,90	1,87
S100-rbs 3	70,5	1,97	1,89
Blank	0,7	0,6	0,45

DNA isolation

	Conc	260/280	260/230
RFP Chloram 1	46,7	1,69	0,79
RFP Chloram 2	33,4	1,68	0,61
RFP Amphi 1	32,8	1,49	0,44
RFP Amphi 2	46,6	1,56	0,47
GFP 1	27,3	1,76	0,7
GFP 2	24,3	1,87	0,79

Testified by

(.....)

Supervisor

(.....)

25 August 2014

Digestion
PART A (EcoR1+Spe1)
- RBS-CheZ 3
- S100-ROSE 1
- T5-RBS 3
- TolC Hifi

PART B (Xba1+Pst1)
- 1018
- GFP
- 1018-HlyA 2

PART B (Nhe1+Pst1)
- HlyBD 1

Mix PART A
- NEB Buffer 3 10ul
- BSA 10X 2ul
- EcoR1 2ul
- Spe1 2ul

Mix PART B
- NEB Buffer 3 7,5ul
- BSA 10X 1,5ul
- Xba1 1,5ul
- Pst1 1,5ul

Single Digest (Pst1) C
- S100-RBS 2
- RBS-GFP 1
- S100-RBS 3
- NEB 7,5
- BSA 1,5
- Pst1 1,5

	Template	DNA	H_2O
A	RBS-CheZ	2,9	13,1
B	S100-Rose 1	2,8	13,2
C	T5-RBS 3	2,7	13,3
D	TolC Hifi	11,3	4,7
E	1018 3	2,6	13,4
F	GFP 4	2,4	13,6
G	1018 HlyA 2	1,7	14,3
H	HlyBD 1 (isolasi)	3,9	10,1
I	RFP 2 isolasi kloram	3	13
J	RFP Amphi kecil 1	1,5	14,5
K	S100-RBS 2	5,8	10,2
L	RBS-GFP	3,4	12,6
M	S100-RBS	3,6	12,4

Testified by Supervisor

(.....) (.....)

27 August 2014

Assembly Step 2
Digest Step 2

A	B
S100-RBS 2	1018
S100-RBS 3	1018
S100-RBS	Nuc HlyA 2
S100-RBS	Nuc HlyA 3
S100-RBS	MalS HlyA 2

	∑ H_2O	DNA
S100-RBS 2	10,2	5,8
S100-RBS 3	12,4	3,6
Nuc HlyA 2	12,6	3,4
Nuc HlyA 3	14,3	1,7
MalS HlyA 2	12,7	3,3
RFP Amphi 1	14,6	1,4

Mix Digest

	A	B	C
NEB 4	7,5 ul	10 ul	2,5 ul
BSA 10X	1,5 ul	2 ul	0,5 ul
EcoR1	1,5 ul	-	0,5 ul
Spe1	1,5 ul	-	-
Xba1	-	2 ul	-
Pst1	-	2 ul	0,5 ul

Ligation Step 2

- S100-RBS – Nuc-HlyA I RFP Amph 5 1 ul Buffer BSA
- S100-RBS – MalS-HlyA I RFP amph 2,0 0,5 ul T4 Ligase
- S100-RBS – 1018 I RFP Amph 2,5 ul ∑ H_2O

Digestion Step 1

	DNA	∑ H_2O
TolC	6,9	9,1
HlyBD	5,9	10,1
HlyBD	3	5

A

- NEB 1 2,5
- BSA 10X 0,5
- EcoR1 0,5
- Spe1 0,5

B

- Neb 1 2,5
- Bsa 10x 0,5

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(.....) (.....)

- Nhe1 0,5
- Pst1 0,5

C

- NEB 1 1,3
- BSA 10X 0,3
- Pst1 0,25

D

- NEB 1 1,3
- BSA 10X 0,3
- Nhe1 0,25

Next Ligation Step 1
TolC-HlyBD

Running
M-TolC-HlyBD total-HlyBD Nhe1-HlyBD Pst1-Nuc HlyA 2-Nuc HlyA 3-Nuc hifi-MalS HlyA-M

Testified by

(.....)

Supervisor

(.....)

28 August 2014

Digestion check Nuc result

	DNA	∑ H_2O
1. Hifi HlyA	3,6	12,4
2. HlyA 4	9,1	6,9
3. Nuclease (stock 1)	3	13
4. Nuclease Hifi	1,9	14,1

PCR colony Electrophoresis
A1 S100-Rose II GFP 1
A2 S100-Rose II GFP 2
B1 RBS_CheZ II GFP 1
B2 RBS_CheZ II GFP 3
C1 S100-Rose II 1018 1
C2 S100-Rose II 1018 2
.....
C10 S100-Rose II 1018 10
D1 T5-RBS II 1018 1
D2 T5-RBS II 1018 2
.....
D10 T5-RBS II 1018 10

Testified by

(.....)

Supervisor

(.....)

29 August 2014

Running Elektroforesis
Marker – RFP Backbone 2 – HlyA –Nuclease –Nuc HlyA 3 – RFP Amphi 1

Nanodrop DNA isolation 28/8/14 (chloramphenicol)

	Concentration	260/280	260/230
T5-RBS II 1018 2	252,2	1,91	1,29
T5-RBS II 1018 3	60,2	2	1,19
T5-RBS II 1018 4	66,4	1,97	1,03
T5-RBS II 1018 7	82	2,06	1,46
S100-Rose II 1018 2	56,1	1,99	1,56
S100-Rose II 1018 5	47,1	2,07	1,13
S100-Rose II 1018 6	39,8	2,09	1,01
S100-Rose II 1018 8	72,7	2,02	1,35
RBS – CheZ II GFP 1	57,7	2,04	1,19
RBS – CheZ II GFP 2	17	2,22	0,73
RBS – CheZ II GFP 3	20,5	1,14	0,72
S100-Rose II GFP 1	151	2,19	1,83
S100-Rose II GFP 2	83	2	1,55

Testified by

(.....)

Supervisor

(.....)

30 August 2014

Running Isolation result
Control 1018-T5 RBS 1018 2-T5 RBS 1018 3-T5 RBS 1018 4-T5 RBS1018 7-S100 Rose 1018 2-S100 Rose 1018 5
S100 Rose 1018 6-s100 Rose 1018 8-S100 Rose GFP 1-S100 Rose GFP 2-RBS CheZ GFP 1

Double digest 1 September 2014 ½ Reaction

			½ DNA	½ H_2O
1. T5-RBS - 1018 3	NEB 4	7,5 ul	2,1	5,9
2. T5-RBS – 1018 4	BSA 10X	1,5 ul	1,9	6,1
3. S100-Rose - 1018 8	EcoR1	1,5 ul	1,8	6,1
4. S100-RBS – 1018 2	Pst1	1,5 ul	1,5	6,5
5. RBS – CheZ-GFP 1	@ 2ul		2,2	5,8

Running
1. T5-RBS (digestion)
2. T5-RBS 1018 3
3. T5-RBS 1018 4
4. HlyA (digestion/hifi)
5. S100-Rose-1018 8
6. S100-RBS-GFP 2
7. RBS-CheZ-GFP 1

Testified by

(.....)

Supervisor

(.....)

1 September 2014

Loading Dye 6X dan LD 10X
M1.V1=M2.V2
10XLD.X=6XLD.60
10X=360
X=36

10XLD.X=6XLD.100ul
10X=600ul
X=60ul
100-60ul = 40TE

Making Ladder DNA (marker)

1. Gone Ruler Mix 50ug = 10ul
2. Loading Dye 6x = 20ul
3. TE 1X Buffer = 70ul +
   = 100 ul

Agenda Lab 1 September 2014

1. Nanodrop Subtilis → Running
2. Check Phenol in fumehood
3. (PCR Mix → Hotstar → Template B.Substilis) 5x reaction → touchdown overnight
4. Innoculate P1018 → Column 3 → 20ml
5. Innoculate T5 RBS 1018 3, 4 → 20ml
6. Digestion → running electrophoresis
7. Check biofilm

Innoculate B. Substilis, P. Aeruginosa, S. Auresus, E. WT, V. cholerae 20 ml

Check LB Broth
Make Plate Agar Stock

Running Electrophoresis 1 Sep 2014
M-T5 RBS-T5 RBS 1018 4-T5 RBS 1018 3-S100 Rose 1018 8-S100 Rose GFP 2-RBS CheZ GFP 1

Using 0,2-2 ul micropipet was not optimal, so we worry that the digestion was not prefect

HotStar PCR
Touchdown
Gradient Temp. Column

1. 1 50^{O}C
2. 3 54,7 ^{O}C
3. 5 55,5 ^{O}C
4. 7 61,8 ^{O}C

Testified by Supervisor

(.....) (.....)

5. 10 68,4 ^{O}C

MIX
∑ H_2O = 31,63 ul
10x taq buffer = 5,5 ul
5x Qsol 11ul
10mM dNTP mix 1,1 ul
Taq HotStar 0,28 ul

PRIMER
Substilisin F 1,375 ul
Substilisin R 1,375 ul

TEMPLATE
Genome 1/3 EB 5ul

MIX ALIQUOT 5 tube PCR → PCR Touch Down HotStar

Nanodrop Genome B. Substilis in1/3 EB

	Concentration	A260	A280	260/280	260/230
Blank	0	1	-0,002	0,17	-0,05
1/3 EB B.subs 1	424	8,481	4,716	1,8	1,74
1/3 EB B.subs 2	306,5	6,129	3,442	1,78	1,76

Testified by Supervisor

(.....) (.....)

2 September 2014

Running Electrophoresis
Substilis 1,2,3,4,5-Marker-HlyA-T5 RBS 1018 3- T5 RBS 1018 4-T5 RBS-S100 Rose 1018 8-S100 Rose GFP 2-RBS CheZ GFP 1

Ligation
S100-RBS 3 II Nuc-HlyA 2 → 16 ^{O}C overnight
S100-RBS 3 II Nuc-HlyA 2 → 23 ^{O}C 1 hour
S100-RBS 3 II MalS-HlyA 2 → 16 ^{O}C overnight
S100-RBS 3 II MalS-HlyA 2 → 23 ^{O}C 1 hour

Transform
1. Kont (-) → SOC <<
2. Kont (+)→ isi RFP Amphi 1
3. S100-RBS 3 II Nuc-HlyA 2 (23 ^{O}C)

To Do List
Electrophoresis → staining
Ligase (AC, heatblock)
Transformation
Isolation

Testified by

(.....)

Supervisor

(.....)

3 September 2014

Take out the transformation
Making the replica
PCR colony, inoculate in to the falcon, 20ml LB Broth
Ligase 16 ^{O}C overnight → heatkill 80 ^{O}C for 20 minute transform

Take out the transformation, replicate S100 RBS Nuc HlyA → template
PCR mix, transform 16 ^{O}C start → put the mix in ice for 60 minute
Digest → ligase (30 ^{O}C 1 hour, 88 ^{O}C 20 minutes)
Take out the transformation from ice and start PCR
Ligase (23 ^{O}C 1 hour, 88 ^{O}C 20 minutes)
Transform 60 ^{O} → 1 hour in ice

Digestion

	Eco + Spe= MalS	Xba+Pst= HlyA	Eco+Pst=RFP 2 chloramphenicol
NEB 4	2,5	2,5	2,5
BSA 10X	0,5	0,5	0,5
Enzim 1	0,5	0,5	0,5
Enzim 2	0,5	0,5	0,5
DNA	2	3,6	3
∑ H_2O	14	12,4	13

Transform : S100-RBS-Nuc-HlyA ligase overnight

Nanodrop

Sample	CONC	260/280	260/230
1018 3	30,1	2,07	1,44
T5 RBS 1018 3	33,1	2,17	1,69
T5 RBS 1018 4	37,4	2,06	1,39

Digestion

	½ DNA	½ H_2O	
1018 -3 (2 sept)	4,2	3,8	NEB 1,25 BSA 0,25 EcoR1 0,25
T5-RBS-1018 3 (2 sept)	3,8	4,2	NEB 3 5ul BSA 10X 1ul EcoR1 1ul Pst1 1ul @2 ul
T5-RBS-1018 4 (2 sept)	3,4	4,6	
T5-RBS-1018 4 (28 august)	2	6	

Running
M-HlyA-MalS-RFP 2-Sub1-Sub2-T5 RBS 1018 4(28Agt)-T5 RBS 1018 3(2 Sept)- T5 RBS 1018 4(2 Sept)- T5 RBS 1018 4(28 August)

Testified by Supervisor

(.....) (.....)

6 September 2014

a. TolC HlyBD
b. MalS HlyA
c. T5 rbs 1018

Nanodrop Isolation Result

Sample	Concentration	A260/280	A260/230
Kontrol (-)	10,8	0,84	0,55
S100 RBS Nuc HlyA 1	67,3	2,04	2,05
S100 RBS Nuc HlyA 3	52,9	2,08	1,8
S100 RBS Nuc HlyA 4	57,6	2,03	1,64
S100 RBS Nuc HlyA 5	58,5	2,09	1,77

Digestion (double cek)

	½ DNA	½ H_2O
S100 RBS Nuc HlyA 1	2	6
S100 RBS Nuc HlyA 3	2,4	5,6
S100 RBS Nuc HlyA 4	2,2	5,8
S100 RBS Nuc HlyA 5	2,2	5,8

NEB 3 1,25
BSA 10X 0,25
EcoR1 0,25
Pst1 0,25

Running
M-T5 RBS 3-T5 RBS 1018 3-T5 RBS 1018 4-RFP Amphi-S100 Rose GFP-M
M-S100 RBS Nuc HlyA 1-S100 RBS Nuc HlyA 3- S100 RBS Nuc HlyA 4- S100 RBS Nuc HlyA 5-Nuc HlyA 2-M

Testified by

(.....)

Supervisor

(.....)

7 September 2014

Nanodrop 1/3 EB Bacillus (dilution 1/50)

	Concentration	260/280	260/230
Blank	0,3	0,94	0,38
1/3EB Bac 1	8,2	2,13	2,08
1/3 EB Bac 2			

Nanodrop 1/3 EB Bacillus 1/10 dilution

	Concentration	260/280	260/230
1/3 EB Bac 1 (1/10)	44,5	1,72	1,7
1/3 EB Bac 2 (1/10)	27,9	1,79	1,64
Blank	0,4	1,5	0,33

Testified by

(.....)

Supervisor

(.....)

8 September 2014

Digestion (double) → for check list

	½ DNA	½ H_2O
S100-RBS-Nuc-HlyA 1	2	6
S100-RBS-Nuc-HlyA 3	2,4	5,6
S100-RBS-Nuc-HlyA 4	2,2	5,8
S100-RBS-Nuc-HlyA 5	2,2	5,8

Mix
NEB 5
BSA 10X 1 @2
Eco 1
Pst 1

Testified by

(.....)

Supervisor

(.....)

9 September 2014

DNA isolation result 8/9/14

	Concentration	260/280	260/230
S100-RBS-Nuc-HlyA 1	39,4	2,06	1,69
S100-RBS-Nuc-HlyA 3	57,3	2,01	1,59
S100-RBS-Nuc-HlyA 4	70,9	1,99	1,91
S100-RBS-Nuc-HlyA 5	41,6	1,97	1,72
Blank	0,3	0,57	0,23

Digestion (double) DNA isolation 8/9/14

	½ DNA	½ H_2O
S100-RBS-Nuc-HlyA 1	3,2	4,8
S100-RBS-Nuc-HlyA 3	2,2	5,8
S100-RBS-Nuc-HlyA 4	1,8	6,2
S100-RBS-Nuc-HlyA 5	3	5

Testified by

(.....)

Supervisor

(.....)

10 September 2014

V1.M1=V2.M2
50.M1=500 . 0,5
M1 = 5 mL

ElectrophoresisRunning
Marker – SNRH / PCR Colony 1 2 3 4 5 6 7 8 – Peptide 1018 Hifi – Digesti RFP Amphi 1018 Eco Pst – Marker

Extraordinary occurence:
Running progress was not finished yet, running was only in the chamber.
Agarose was taken out from the chamber, so we worry that will soften the agarose if it was soaked overnight in the TAE chamber

Testified by

(.....)

Supervisor

(.....)

11 September 2014

Running electrophoresis

SNRH 1 2 3 4 5 6 7 8 – Marker – Substilisin Hifi (51 is genom, 51 HS, 54 HS) – Peptide 1018 Hifi 1 + 5

✓ Make LB Broth 400 mL
✓ Make LB agar 300 mL → 15 petri dish [4 antibiotic (-), 10 (7 chloramphenicol, 3 amphicilin)]
✓ Making stock: amphicilin 6, chloramphenicol 3, without antibiotic 2
✓ Electrophoresis → making new agar
✓ Innoculate SNRH
✓ PCR PFX Hifi 10 → Substilis 51 ^{O}C, 5 → 1018 53 ^{O}C
✓ Innoculate 6 pathogen bacteria → 15 mL

Prepare
6 falcon (1 falcon control)

Nanodrop Substilis Hifi			
Concentration	10,3 ng/ul		11,1
260/280	2,28	→ heat	2,01
260/230	0,92		11,04

Testified by

(.....)

Supervisor

(.....)

12 September 2014

Ligase

		H_2O	Mix Ligase
1018 2	Amphi 2	4,5	5 ul buffer 3 2,5 ul T4 Ligase 1,5 1,5 ul @1,5 @
TolC 2-HlyBD 2	Amphi 2	2,5	
S100 RBS 2 Nuc HlyA 2	Amphi 2	2,5	
MalS 2- HlyA 2	Amphi 2	2,5	

Testified by

(.....)

Supervisor

(.....)

13 September 2014 → Yesterday transform result is too little.

- Repeat the transform (left 50 ul, right 200ul)
  1018-AMPHI
  S100-RBS-N H
  MalS-HlyA
- Grow the biofilm
- Autoclave SOC

Running Substilisin

1. 45,3 ^{O}C
2. 45,9 ^{O}C
3. 46,7 ^{O}C
4. 47,8 ^{O}C
5. 49,3 ^{O}C
6. 551 ^{O}C
7. 52,4 ^{O}C
8. 53,5 ^{O}C
9. 54,3 ^{O}C

Testified by

(.....)

Supervisor

(.....)

15 September 2014

iGEM lab schedule

1. PCR HotStar Substilisin
2. Take out the replica
3. Running PCR Colony Running Hifi Result
4. Innoculate PCR Good Colony, there's starter culture
5. If the running isn't good→ ligase again with TolC-HlyBD and the other unwell parts
6. Ligation of TolC HlyBD
   Ligation :
   TolC 1,6ul
   HlyBD 4ul
   RFP 2ul
   Buffer 1ul
   Ligase 0,5ul
   Air 0,9ul

   PFX –
   PCR =
   PCR HS → ?

1. Running PCR Colony
   Marker – (Amphi-P1018 1 2 3 4 5) – (MalS-HlyA 1 2 3 4) – (S100-RBS-Nuc-HlyA 1 2 3)
2. Pcr HotStar Substilisin
3. Ligation
   - TolC : HlyBD : amphiRFP = 1,6 : 4 : 2 ∑ H_2O 0,9
   - S100-RBS: Nuc HlyA : amphi RFP = 0,2 : 0,8 :2 ∑ H_2O 5,5
4. Innoculate
   - 1018 Amphi 1 & 5
   - MalS-HlyA 2 & 3
   - S100 RBS Nuc HlyA 2 & 3 (from replica 3/9/14)
   - S100 RBS Nuc HlyA 1 & 3 (from replica 14/9/14)
5. Change the Broth (bacteria's biofilm)
6. Running HiFI Substilis Purif & 1018 Hifi

Running electrophoresis 15 Sept 2014
1018 Amphi 336
S100 RBS Nuc HlyA
35 12 561 186 + 300 +1094

MalS HlyA
2067 186 + 300 = 2553

Testified by Supervisor

(.....) (.....)

16 September 2014

Lab iGEM

1. DNA isolation of Running HS
2. Digest, Running → SNRH, MalS HlyA, P1018 Amp, SNRH Rose
3. LMA → PCR PFX P1018
   PCR HS Substilisin
4. Staining biofilm
5. Transform → spread (amphicilin)

Plate 2 Well 3F Chloram S100+RBS Kit

To do list target :

1. LMA P1018 PCRPFX Hifi : delayed → 2%
   LMA Substilisin PCR Hotstar : delayed → 1,5%

2. Isolasi :
   1018-Amphi 1 & 5
   MalS-HlyA 2 & 3
   S100-RBS-Nuc-HlyA 2 & 3 (from replica 3/9/14) → there's band
   S100-RBS-Nuc-HlyA 1 & 3 (from replica 14/9/14) → there's no band

3. Nanodrop
   P1018 PCR PFX Hifi unpurified
   Substilisin PCR PFX Hifi purified
   Substilisin Hotstar unpurified

   Running
   Marker – PCR HotStar Substilisin

Nanodrop

Sample	Concenration	A260	A280	260/280	260/230
PCR HS Substilisin	3,6	6,673	5,621	1,19	1,43
	131,5	2,627	1,413	1,86	0,34
	135,3	2,709	1,453	1,86	0,24
PCR PFX Hifi 1018	179,5	3,469	1,889	1,84	2,05
	169,8	3,395	1,848	1,84	2,2

Testified by Supervisor

(.....) (.....)

17 September 2014

Nanodrop

		∑ H_2O	Conc	DNA	260/280	260/230
	K (-)		0,7			
1	SNRH 1 (14)	4,9	94,6	1,5	1,98	1,96
2	SNRH 2 (3)	4,61	83,6	1,79	1,94	1,99
3	SNRH 3 (14)	4,8	93,5	1,6	2,12	1,65
4	SNRH 3 (3)	2,26	36,2	4,14	2,3	1,64
5	1018 Amp 1	4,75	90,8	1,65	2,09	1,9
6	1018 Amp 5	4	73,7	2	2,08	1,99
7	MalS HlyA 2	3,38	49,6	3,02	1,96	1,86
8	MalS HlyA 3	1,05	28	5,35	2,06	1,46

18 September 2014

List of Digestion

29 August 2014	RBS CheZ II GFP 1	1,39	57,7	2,5	2,09	1,19

Testified by

(.....)

Supervisor

(.....)

18 September 2014

Digest the isolation of RBS CheZ GFP ✓ → Running ✓
Take out the transforman ✓ → Innoculate ✓
PCR Hotstar substilis / evening ✓
Innoculate the transforman S100 RBS Device / evening ✓
Make the bacteria stock -80^{O}C + streak → pathogen's biofilm / evening ✓
Take out the inoculation from incubator shaker ✓
Evening

Running PCR Colony
Marker – SNRH 1 2 3 4 5 6 – TolC HlyBD 1 2 –Hotstar 1 2 – Marker

M – SNRH 1(14) - SNRH 3(14) - SNRH 2(3) - SNRH 3(3) – 1018 Amphi 1 5 – MalS HlyA 2 3

Repeat the innoculate the TolC-HlyBD

Nanodrop

	Concentration	A260/280	260/230
SNRH 3 (from replica 16/9/14)	65,4	2,02	1,96
Blank	0,2		

Innoculate S100-RBS

Testified by

(.....)

Supervisor

(.....)

19 September 2014

1. Running PCR Substilis
2. Isolation
    - TolC HlyBD 1A
    - TolC HlyBD 1B
    - S100 RBS 1
    - S100 RBS 2
    - S100 RBS 3
3. Take out the pathogen bacteria
4. Digestion (repeat) + (new 5)

		6,4 ul		DNA 150 ng MIX ½ Reaction (V=8ul) NEB Buffer 1,5 ul (@1ul) BSA 10X 3 ul (@0,2 ul) EcoR1 3 ul (@0,241) Spe1 3ul (@0,241) @1,6 ul
		DNA	∑ H_2O	
1	SNRH 1 (14/9)	2	4,4	
2	SNRH 2 (3/9)	2	4,4	
3	SNRH 3 (14/9)	2	4,4	
4	SNRH 3 (3/9)	2,3	4,1	
5	1018 Amp 1	2	4,4	
6	1018 Amp 5	2	4,4	
7	MalS HlyA 2	3	3,4	
8	MalS Hlya 3	3,4	1	
9	RBS CheZ GFP	2,5	3,9	
10	TolC HlyBD 1A	1,8	4,6	
11	TolC HlyBD 1B	0,7	5,7	
12	S100 RBS 1	2	4,4	
13	S100 RBS 2	1,8	4,6	
14	S100 RBS 3	3	3,4	
15	SNRH 3 (16/9)	2,3	4,1	

5. PCR Substilis
6. Nanodrop

	Concentration (ng/ul)	A260/280	260/230
Blank	-0,1		
TolC HlyBD 1A	102,4	1,95	2,05
TolC HlyBD 1B	288	1,9	2,11
S100 RBS 1	92,5	1,95	2,02
S100 RBS 2	105,2	1,94	2,13
S100 RBS 3	59,5	1,98	2

Testified by Supervisor

(.....) (.....)

20 September 2014

Digestion (assembly) 150 ng

				∑ H_2O	DNA
	[	S100	K	7,96	2,04
		MalS HlyA 2	Chlor	7	3
A		SNRH 1 -14	K	8,42	1,58
B	[	T5 RBS	K	8,4	1,6
C		1018 Amphi 5	K	8	2
		RFP chloram		8,2	4,8

TH 1 A, TH 1B, PA, PAS, MN 2, MN 3, SR 2, SR3
SN2-3, SN3,3 , SN3-6, SN3 14, SN1-14, SR, RC

Testified by

(.....)

Supervisor

(.....)

22 September 2014

1. Single Digest
   TolC HlyBD 1A 125 ng
   ∑ H_2O: 6,8
   ½ DNA: 12
   NEB Buff: 1,25
   BSA 10X: 0,25
   Xba I : 0,25

2. Ligation
   1. S100 - MalS HlyA – RFP chloramphenicol
      0,03 2,18 2
      0,5 2,18 2 ∑ H_2O 3,8
   2. T5 RBS – 1018 – RFP chloramphenicol
      - T5-RBS
        (138/2070)*25=1,6
        (1,6/25)*2ul=0,13 ul
      - 1018
        (36/2070)*25 = 0,03 ul

      T5 RBS – 1018 – RFP chloramphenicol
      0,13 0,03 2
      0,5 0,5 2 ∑ H_2O 5,5, Buffer T4 3uL, T4 Ligase 1,5 uL @ 1,5 uL
3. SNRH – RFP chloramphenicol
   (796/2070)*25=9,5

   Buff T4 3ul
   T4 Ligase 1,5 ul
   @1,5 ul

LIGATION

(Insert length / vector length)*vector mass(ng) = insert mass (ng)

Molar ration 1:1
Vector 25 ng/2 ul

Example:
TolC –HlyBD –RFP
1500 4000 2000
1,5 4 2

TolC → (1500/2070)*25 = ... ng
(.....ng/25)*2ul = ... ul

Testified by Supervisor

(.....) (.....)

HlyBD →

S100 MalS HlyA

S100 : (35/2070)*25=10,42
(A/25)*2ul = 0,03 ul
MalS HlyA : (2253/2070)*25=27,21 ng
(27,21/25)*2ul=2,18ul

S100 MalS HlyA RFP chloramphenicol
0,03 2,18 2
1,5

PCR Substilis HotStar (51, 53)
Template Bacillus 1/10

Running Hasil Double Digest kemarin(20/9/14) and Single digest TolC HlyBD (22/9/14)

TolC HlyBD non digest – TH 1A single digest – TH 1B double digest – TolC Hifi – TolC double digest 12/9 – HlyBD double digest 12/9 (Nhe Pst)

S100-T5 RBS – MalS HlyA – 1018 Amp – SNRH –RFP Chloramphenicol

TolC = (1539/2070)*2=1,5ul
HlyBD=(4000/2070)*2=3,4 ul
RFP Amp = 2ul
∑ H_2O = 1,1

Testified by Supervisor

(.....) (.....)

24 September 2014

1. Take out the transforman → in fridge for 1 hour 11.00 → incubator 12.00
2. Make LB Broth + LB agar
   New Tips (5 boxes)
3. Inoculate replica on 12.00 o'clock
4. Revitalize bakteri 10.15 a.m
5. Innoculate bacteria transforman 12.00 a.m

Testified by

(.....)

Supervisor

(.....)

25 September 2014

1. Running PCR Colony
   MH1-MH2-SN1-SN2-SN3-SN4-SN5-T181-T182-T183-T184-T185
2. Isolation
   T518 1, 2, 4
   SNRH 1, 2, 3

Testified by

(.....)

Supervisor

(.....)

26 September 2014

1. Nanodrop

		Concentration	260/280	260/230
Isolation 25/9/14	SNRH 1	66,9	1,91	1,96
	SNRH 2	44,6	1,90	2,07
	SNRH 3	79,9	1,91	2,07
	T518 1	58,7	1,89	2,04
	T518 2	53,7	1,95	2,06
	T518 4	62,4	1,87	1,9
	Blank	0,7	0,67	0,52

2. Ligation
   - TolC HlyBD
   - MalS HlyA
3. Digestion

	6,4 ul		MIX
	∑ H_2O	DNA	NEB Buffer 3 6ul
SNRH 1	2,4	3	BSA10X 1,2ul
SNRH 2	2	4,4	Eco 0,6ul
SNRH 3	3,9	2,5	Pst 0,6ul

Nanodrop Result DNA Isolation September 26th 2014

	Concentration	260/280	250/230
Blank	2		
1018 3	34,1	1,96	2,23
RFP Amphi	26,5	2,16	1,78
SNRH 3	43,3	2	1,74
MalS HlyA	28,7	1,96	1,69
Tr 1018 1	18	1,94	1,43
Nuc HlyA	21,8	2,09	1,36

Running Electrophoresis List

MalS HlyA Insert
Backbone RFP Amphi
MalS HlyA
R 1018 1
RFP Amphi
1018 3
Nuc HlyA
SRNH

Testified by

(.....)

Supervisor

(.....)

29 September 2014

	Digestion	DNA 150	∑ H_2O
1	1018-3	4,4	2,2
~~2~~	TR 18	6,6	0
~~3~~	NH	6,6	0
4	SRNH-3	3,5	3,1
5	MH Amphi	5,2	1,4
6	RFP Amphi	5,5	1,1
		DNA 200	∑ H_2O
7	MalS HlyA 2	4	2,6
8	RFP Chloramphenicol	2,4	4,2

Mix

NEB 3	@ 1 uL	x 6 =	6 uL
BSA 10 X	@ 0,2 uL	x 6 =	1,2 uL
EcoR I	@ 0,1 uL	x 6 =	0,6 uL
Pst I	@ 0,1 uL	x 6 =	0,6 uL
Total	@ 1,4 uL		7,4 uL

Testified by

(.....)

Supervisor

(.....)

PCR Touchdown Substilis

1. 50
2. 50,3
3. 50,9
4. 51,7
5. 52,8
6. 54,3
7. 36
8. 57,9
9. 58,5
10. 39,3

Digestion Receipt 30/9/2014
Mix

NEB 3	@ 1 uL	x 11 =	11 uL
BSA 10 X	@ 0,2 uL	x 11 =	2,2 uL
EcoR I	@ 0,1 uL	x 11 =	1,1 uL
Pst I	@ 0,1 uL	x 11 =	1,1 uL
Total	@ 1,4 uL		15,4 uL

Rumus DNA 150- $\sum H_2O$
Volume template = 150/concentration = x
Volume $\sum H_2O$ = 6,0 – x = y
x + y = 6,6 uL

Total per tube = mix 1,4 uL + 6,6 uL = 8 uL

Testified by

(.....)

Supervisor

(.....)

Marker – 1018 3 x x x x – SNRH – Marker – MalS HlyA – RFP Chloram – RFP Amphi – MH Amphi

Nanodrop DNA Isolation 30/9/2014

No	Sample	Concentration	260/280	260/230	x	y (∑ H_2O)
1	Blank	0,1	1,02	0,09		
2	T5 RBS 1018	44,8	1,92	2,11	3,35	3,25
3	1018 3	76,5	1,94	2,28	1,96	4,64
4	SNRH 3	104,7	1,89	2,2	1,43	5,17
5	S100 MalS HlyA 4	161,0	1,88	2,25	0,93	5,67
6	S100 MalS HlyA 5	142,1	1,88	2,25	1,05	5,55
7	TolC HlyBD 2	173,1	1,88	2,16	0,86	5,74
8	TolC HlyBD 4	96,7	1,89	2,08	1,55	5,05
9	Nuc HlyA 2	55,4	1,94	1,88	2,7	3,9
10	RFP Amphi	27,8	2,01	1,2	5,39	1,21
11	TolC HlyBD 3	335,1	1,89	2,21	0,45	6,15
12	MalS HlyA 2	72,7	1,9	1,37	2,06	4,54

Testified by

(.....)

Supervisor

(.....)

1 October 2014

iGEM Lab Schedule

1. PCR Substilis 10 reaction scale up

Mix	Primer mix = 5,25
DW 60,38	Sub F = 2,625
SQsol 21,0	Sub R = 2,625
10 x taq 10,5	Taq 0,53
MgCl 2,1	Template 5,25
dNTP 2,1	

   Annealing Temperature $51^{O}C$

2. PCR Colony Replica 11 Reaction

PCR Tube Code	PCR Coloby Replica
A	Sp100-Rose 1
B	Sp100-Rose 2
C	Sp100-Rose 3
D	Sp100-Rose-1018 1
E	Sp100-Rose-1018 3
F	Sp100-Rose-1018 5
G	Sp100-Rose-1018 6
H	Sp100-Rose-1018 8
I	Sp100-Rose-1018 10
J	RNA rose
K	RNA rose

   PCR Colony transforman MalS HlyA 6 colonies
   Make the replica

Testified by Supervisor

(.....) (.....)

Repeat the MalS HlyA 2 digestion

EcoRI = 0,1 uL
PstI = 0,1 uL
NEB 3 = 1 uL
BSA 10x = 0,2 uL
DW = 6,6 – 2,06 = 4,54

Template DNA = 150/72,7 = 2,06

Bikin LB Agar 20 Petri Dishes
6 non antibiotics
4 amphicilin
10 chloramphenicol

Electrophoresis Running

Testified by

(.....)

Supervisor

(.....)

3 October 2014

	Digestion	250 ng DNA	∑ H_2O	NEB @ 2 Enzyme @ 0,2 Enzyme @ 0,2
1	T5-RBS 3	2,7	5,3	
2	TolC HlyBD	0,75	7,25	
3	RFP Chloramphenicol	1,5	6,5	

0,238 gram/mL → 1M

Testified by

(.....)

Supervisor

(.....)

5 October 2014

Ligation

T5 RBS	1 uL	T4 Buffer	1 uL
TolC HlyBD	4,8 uL	T4 Ligase	0,5 uL
RFP chloram	2 uL		
Total	7,8 uL	Total	1,5 uL

TOTAL 7,8 + 1,5 = 9,3 uL + 0,7 uL H_2O = 10 uL

1. Ligation T5-RBS II TolC-HlyBD → transform ✓
2. Isolation, nanodrop, running ✓

		Concentration	A260/280	260/230
S Rose 2	K	54,1	1,99	2,12
S Rose 1018 1	K	41,6	1,91	1,85
MalS HlyA 2	K	121,5	1,9	2,18
MalS HlyA 3	K	112,6	1,91	2,09
MalS HlyA 5	K	142,9	1,89	2,16

3. PCR Substilisin, Running ✓
   Running: Marker – PCR Substilisin 1 2 3 4 5 6 7 8 9 10 – Marker

4. Make LB Broth and SOB Media 500 mL (without $MgCl_2$) ✓
5. Stock reuse microtube 1,7 mL & new one for induction ✓
   Stock cold tips for making competent cell
6. Digestion of Isolation✓

		200 DNA	∑ H_2O	NEB Buffer @ 2 x 5 = 10 EcoRI @ 0,2 x 5 = 1 Pst I @ 0,2 x 5 = 1
1	SR 2	3,7	3,9	
2	SH 8	4,8	2,8	
3	MH 2	1,7	5,9	
4	MH 3	1,8	5,8	→ Running electrophoresis
5	MH 5	1,5	6,1	✓ Marker-SR181-SR2-MH2-MH3-MalS-Marker

7. Innoculate
   - 14/09 SNRH 3 (K) 15 mL
   - T5 RBS 1018 1 (K) 15 mL
   - S100 MalS HlyA 4 (K) 15 mL
   - Nuc HlyA 2 (K) 15 mL
   - MalS HlyA 2 (A) 15 mL
   - Pirare (K) 10 mL
   - BL21(-AB) 10 mL
   - Top10 (-AB) 10 mL
   - DH5 alpha (-AB) 10 mL

Testified by

(.....)

Supervisor

(.....)

6 October 2014

- Prepare the sample for submit
- Make competent cell
  BL21
  DH5 alpha
  Pirare
  Top10

No Tube	Nanodrop	Concentration	A260	A280	260/280	260/230
21	Reverse Cqss	116,9	3,339	1,613	2,07	2,5
22	Reverse Cqss	114,3	2,286	1,080	2,12	2,08
23	Reversed ybcl	125,4	2,509	1,180	2,13	2,08
24	Reversed ybcl	132,9	2,657	1,247	2,13	2,28
25	Reversed yain	79,3	1,586	0,725	2,19	2,62
26	Reversed yain	81,0	1,620	0,733	2,21	2,69

Innoculate biofilm pathogen bacteria 2 well plate

1. Well Plate Non IPTG Gradient Column

	K-	E. coli	V. cholerae	B. substilis	S. aureus	P. aeruginosa	K. pneumonas
1	O	o	O	o	o	o	o
2	O	o	O	o	o	o	o
3	O	o	O	o	o	o	o
4	O	o	O	o	o	o	o

2. Well plate IPTG Gradient Column for Peptide 1018
   K-
   E. coli
   V. cholerae
   B. substilis
   S. aureus
   P. aeruginosa
   K. pneumonas
3. Revitalize bacteria for innoculating to M9 tomorrow 7 October 2014
4. Make M9 Medium

Testified by

(.....)

Supervisor

(.....)

IPTG
V1.M1=V2.M2
V1.500=0,05.200

M2=0

		M2	V2	V1	M1=0,5M=500mM → 50 mM → 10 mM
1	0,2 uL	0,05	200 uL	0,02	
2	0,4 uL	0,1	200 uL	0,04	
3	1 uL	0,25	200 uL	0,1	
4	2 uL	0,5	200 uL	0,2	
5	3 uL	0,75	200 uL	0,3	
6	4 uL	1	200 uL	0,4	
7	5 uL	1,25	200 uL	0,5	
8	6 uL	1,5	200 uL	0,6	
9	7 uL	1,75	200 uL	0,7	
10	8 uL	2	200 uL	0,8	

V1.M1=V2.M2
V1.500mM=1000uL.50mM
V1.5=500
V1=100 uL
1000-100= 900 uL H_2O

V1.M1=V2.M2
V1.50=200uL.10
V1=40 → 160 uL H_2O

For Tomorrow →8 October

IPTG Concentration	M9 Shake Added Volume	
0,05 Mm	199 uL	IPTG Stock has used 10 mM
0,1 mM	198 uL	
0,25 mM	197 uL	
0,5 mM	198 uL	IPTG Stock 50 mM
0,75 mM	197 uL	
1 mM	196 uL	
1,25 mM	195 uL	
1,5 mM	194 uL	
1,75 mM	193 uL	
2 mM	192 uL	
0 mM	200 uL	

Extraordinary occurences
Characterizations activity has encountered obstacle not only biofilm but also enzyme expression.
Details :

- P1018 Characterization
  - ➔ M9 can't be used
  - ➔ Undergo Millard effect
  - ➔ Failure : M9 + Glucose can't be autoclaved "caramelize" → brownish M9
- Wrong receipt for M9
  Evaluation :

Testified by Supervisor

(.....) (.....)

1. Composition and presentage must be written → write the clear information
2. Glucose has not mixed in the beginning + has not autoclaved → filtered
3. There's no sterile bottle → prepare the bottle!

- Can't do spectrofotometri test → sample was not ready yet

Sequencing

T5 RBS Peptide 1018 Sp100 RBS Nuc HlyA Sp100 MalS HlyA	] @ 10ng/uL 8uL + primer forward, primer reverse @ 5 mM 1 uL 2 reaction

Testified by

(.....)

Supervisor

(.....)

1. Clean up the biofilm
2. New innoculation LB Broth and M9
3. Take out the replica
4. Staining

1. 96 well plate was filled with LB Broth 198 uL
2. Add pathogen bacteria 2 uL and incubate overnight in static incubator
3. Well was filled crystal violet 0,5% 200 uL → incubate 10 minute
4. Crystal violet was took out → put in to the bottle = reused crystal violet
5. Wash the well, soak it in the water and wah for 4 times
6. Well is contain crystal violet that pinned in biofilm
7. Well was filled 96% ethanol (v/v) and acetate acid 2%
   96% absolute ethanol + 2% CH_3COOH (v/v)
   96 uL absolute ethanol + 2 uL acetate acid + 2 uL H_2O
8. Wait for 2 minutes → bring it into pass through box
9. Elisa reader

Testified by

(.....)

Supervisor

(.....)

7 October 2014

Make Stock Agar

1. Prepare LB agar from autoclave. LB starch → LB agar and aquades has heated in the oven
   300 mL LB sterile agar + 200 mL sterile aquades
   Starch powder 1,5 gr in alumunium foil put in the pass through box
2. 200 mL aquades → pour in 500 mL bottle glass + 1,5 gr starch powder, heat in the oven until disolve
3. Discard 120 mL. The excess 80 mL starch. Add 120 mL LB agar, then aliquot it to 10 petri dishes (@ 20 mL).
4. Add chloramphenicol 200 ng/uL

Innoculation the bacteria to M9

1. Preapare M9 medium and overnight bacteria culture in LB Broth
2. Take 400 uL bacteria from LB broth, drop into 20 mL M9
   1 control (-) + 5 bacteria :
   T5 RBS P1018 → M9 without glucose
   SRNH
   SMH
   RNH
   MH
3. Put in to the shaker incubator 37^{O}C overnight

Testified by

(.....)

Supervisor

(.....)

8 October 2014

1. Sequencing
   Sample template 8 uL
   - SRNH: S100-RBS-Nuc-HlyA
   - SMH
   - T5 RBS Peptide

   Primer 5 uM @ H_2O → VF2 & VR @ 1uL per reaction
2. LB revitalization
3. 1018 activation

Testified by

(.....)

Supervisor

(.....)

10 October 2014

V1.M1=V2.M2
96.1000=70.X
X=1371

Plate 1 : 4 x 7
LB(-) E. coli – V. Cholerae – B. Subs – S. Aureus – P. Aeuroginosa- K. Pneumonas

Plate 2
LB(-) E. coli – V. Cholerae – B. Subs – S. Aureus – P. Aeuroginosa- K. Pneumonas

Transformation (NEB/T5)

1. Competent cell + gene of interest (10% comp→ ligase 0,5-2 uL)
2. Incubate in ice for 30 minutes
3. Heatshock 55 s
4. Incubate ice $4^{O}C$ for 2 minutes.
5. Add (2x competent cell) SOC
6. Shaker incubation 1 hour
7. Plate M9

Lower cell
Polyacrilamide 30% 1,4 mL = 1400 uL 2800
Tris HCl 1,5 M pH 8,8 0,875 mL = 875 uL 1750
SDS 10% 35 uL
APS 17,9 uL
TEMED 3,5 uL
H_2O

3500 – 2331,4 uL
H_2O = 1168,6 Ul
Tris HCl 6,8 375 uL
SDS 10% 15 uL
Polyacrilamide 200 uL
d H_2O 901 uL
APS 10% 7,5 uL
TEMED 1,5 uL

1,5 total
1,5 1500 uL → 901 uL

Testified by Supervisor

(.....) (.....)

11 October 2014

Characterization

A. Biofilm Degradation
   1. Biofilm growth from 2 days ago (9/10/14) is treated
      - MH SNRH NH
      - SNRH MHX (+)
      - Then, staining
   2. Running SDS Page → overnight
      - From Device at M9

B. Antimicrobial Assay
   - Dilution 1:20 from innoculation M9 (V=5 mL) was turbid (hope OD still high)
     a. 1018
     b. T5 RBS 1018 → in falcon → well plate Tripto → Elisa reader OD 595 nm
   - Give IPTG induction

C. Starch Degradation Assay → MalS
   - Make starch agar 200 mL → 4 gr starch 100 mL, 3,....gr LB agar 100 mL
   - Centrifuge 1,5 mL innoculation result device (E. coli WT, SMH, MH) in LB
   - Take supernatan, drop in to LB and starch agar

E. coli WT	E. coli SMH
E. coli (-)	E. coli MH

     Time variable : 16 hours, 24 hours, 32 hours, 48 hours (?)
   - Take the pellet to LB and pellet from sonication, drop and streak it on the plate
     Pellet → live cell that still have metabolic activity
     →primer metabolism →grow, biner
     →secondary metabolism→enzyme secretion
     Pellet→is worried that will add bias variable
     → because the cell is still live and can produce extracelluler (amylase)
     But, if it is sonicated → pellet → lysis = cell will death
     Sonication = cell will death → there's no primer metabolic activity, secondary.
     Cell was lysis → can check enzyme in cell → the lysis cell is intracelluler without activity variable

Testified by

(.....)

Supervisor

(.....)

12 October 2014

1. Flooding Iodine
   16 Hours [LB agar & LB starch = Supernatan] [LB starch=pelet]
   24 Hours [LB agar & LB starch = Supernatan]

2. SDS Page

Supernatan				Marker	Pellet
SMH	MH	SRNH	NH	SMH	SRNH
M9	M9	M9	M9	M9 1	M9

SMH LB	MH 9	MH LB pelet	SMH LB pelet	Marker

3. IPTG Induction P1018
   Use LB

4. Repeat innoculation 96 well plate (2) → for biofilm
   6 pathogen bacteria

5. Revitalize bacteria
   E. Coli mutant
   SMH MH SRNH NH
   6 pathogen bacteria

Testified by Supervisor

(.....) (.....)

13 October 2014

1. Flooding Iodine 36 hours

2. Innoculate E. coli mutant
   1. MH2
   2. SRNH 4
   3. NH2
   4. SRNH3
   5. P1018 3
   6. T5 RBS P1018 1

   Pathogen bacteria → bacteria revitalization
   1. E. coli
   2. V. Cholerae
   3. Bacillus substilis
   4. Pseudomonas aeruginosa
   5. Staphylocuccus aureus
   6. Kleoseilla pneumoniae

3. Repeat Biofilm Protocol
   - Take out the LB + pathogen bacteria
   - Fill new LB 200 uL + 2 uL pathogen based on protocol

	MH2	SMH4	NH	Plate 1
	1 2 3 4	5 6 7 8	9 10 11 12	
E. coli				
V. cholera				
B. Substilis				
P. aeruginosa				
S. aureus				
K. pneumoniae				
Blank				
LB (-)				

	SRNH	MIX SRNH 3 SMH 4	No E.coli Mutant	Plate 2
	1 2 3 4	5 6 7 8	9 10 11 12	
E. coli				
V. cholera				
B. Substilis				
P. aeruginosa				
S. aureus				
K. pneumoniae				
Blank				
LB (-)				

   -

4. Isolation, digestion

Blank	0,4	1,52	0,67
SMH	31,3	1,99	1,13
SRNP	33,8	2,09	1,47
NH	116,1	1,91	0,85
T5	4	1,94	1,18

Testified by Supervisor

(.....) (.....)

MH	130,8	1,99	0,8
1018	127,4	1,97	1

M9

100 mL M9 Salts	150mL
1mL of 1 M $MgSO_4$ steril	0,75 mL
10 mL → 20% glucose	7,5 mL
50 uL 1M $CaCl_2$	37,5 mL
+ dH_2O → sampai 500 mL	375 mL

$Na_2HPO_4.7H_2O$	16 gr
KH_2PO_4	3,75 gr
NaCl	0,625 gr
NH_4Cl	1,25 gr
$MgSO_4$	1,23 gr
$CaCl_2$	0,73 gr

Testified by

(.....)

Supervisor

(.....)

Testified by	Supervisor
(.....)	(.....)